

Remarks

Claims 25-76 are pending in the subject application. By this Amendment, Applicants have canceled claims 25-51, 57-60 and 66-75 and amended claims 52-54, 56 and 61-65. Support for the amendments can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 52-56, 61-65 and 76 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested.

The application is objected to on the grounds that the subject specification fails to comply with the requirements of 37 CFR §1.821 through 1.825. Specifically, the Office Action indicates that a new sequence listing is required because page 28 of the specification recites sequences with no SEQ ID NO. In addition, claims 31, 33, 35, 43, 45, 47, 70, 72 and 74 are objected to for reciting a sequence without a sequence identifier. By this Amendment, Applicants have amended page 28 of the specification and the objected claims to indicate the sequence identifier number associated with each sequence. In addition, a Submission of Sequence Listing Under §1.821, including a replacement sequence listing on paper and a computer readable format, is attached. Accordingly, reconsideration and withdrawal of the objections is respectfully requested.

Claims 25-51 are rejected under 35 U.S.C. §103(a) as obvious over Pawlowski *et al.* (1997) in view of Sharma *et al.* (U.S. Patent No. 5,594,115). The Office Action argues that it would have been obvious to one of ordinary skill in the art to use the DNA construct comprising an isolated polynucleotide sequence encoding AgNt84 polypeptide taught by Pawlowski *et al.*, and to modify that construct by incorporating two or more of the polynucleotides in a multimeric form, separated by linker sequences, to produce chimeric polynucleotides encoding fusion polypeptides separated by cleavable linker peptides as taught by Sharma *et al.* to produce recombinant fusion proteins and purifying them with immobilized metal ions. The Office Action further argues that one skilled in the art would have had a reasonable expectation of success as taught by Sharma *et al.* and that Applicants have not shown any unexpected results associated with the use of multiple polynucleotide sequences encoding SEQ ID NO: 1, 3 or 4 in the fusion construct. Applicants respectfully assert that the claimed invention is not obvious over the cited references. However, in the interest of advancing prosecution in this matter, Applicants have canceled these claims thereby rendering this issue moot.

Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

Claims 52-76 are rejected under 35 U.S.C. § 112, first paragraph, as nonenabled by the subject specification. The Office Action argues that the as-filed specification does not provide guidance for a transformed plant expressing a polynucleotide encoding an exemplified or non-exemplified polynucleotide sequence encoding the polypeptide sequence of SEQ ID NO: 1, 3, or 4 that is capable of phytoremediation of a contaminated site. The Office Action also notes that SEQ ID NO: 4 is a metal binding domain, that SEQ ID NO: 3 is a signal peptide and that Applicants have not disclosed a single transformed plant having a phytoremediation property as a result of expressing a polynucleotide encoding SEQ ID NO: 4, or a polynucleotide encoding SEQ ID NO: 3 operably linked to a heterologous metal binding polypeptide. Therefore, the specification provides no more than an invitation to experiment, thus requiring extensive and undue experimentation. Applicants respectfully assert that the claims as filed are enabled and traverse the rejection of record.

Enablement is a legal determination of whether a patent enables one skilled in the art to make and use the claimed invention (*Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 960, 220 U.S.P.Q. 592, 599 (Fed. Cir. 1983)) and is not precluded even if some experimentation is necessary. *Atlas Powder Co. v. E.I. Du Pont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 U.S.P.Q. 409, 413 (Fed. Cir. 1984); *W.L. Gore and Associates v. Garlock, Inc.*, 721 F.2d 1540, 1556, 220 U.S.P.Q. 303, 315 (Fed. Cir. 1983). Applicants also submit that nothing more than objective enablement is required, and therefore, it is irrelevant whether this teaching is provided through broad terminology or illustrative examples (compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, does not turn on whether an example is disclosed (see M.P.E.P. §2164.02)). Indeed, the Federal Circuit has held that “The mere fact that something has not previously been done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it.” *Gould v. Quigg*, 822 F.2d 1074, 1078, 3 U.S.P.Q. 2d 1302, 1304 (Fed. Cir. 1987) (quoting *In re Chilowsky*, 229 F.2d 457, 461, 108 U.S.P.Q. 321, 325 (C.C.P.A. 1956)). Additionally, the Patent and Trademark Office Board of Patent Appeals and Interferences has stated: “The test [for enablement] is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the

direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed". *Ex parte Jackson*, 217 U.S.P.Q. 804, 807 (1982); *see also Ex parte Erlich* 3 U.S.P.Q.2d 1011 (B.P.A.I. 1982) (observing that although a method might be "tedious and laborious," such experimentation is nevertheless "routine" defining "routine" experiments as those which use known methods in combination with the variables taught in the patent to achieve the expected, specific, patented result).

Turning to the rejection of record, Applicants submit that the presently claimed invention is enabled by the teachings of the as-filed specification. The Office Action cites various references indicating that the ability of a plant to accumulate heavy metals is genotype dependent and varies greatly between species and between cultivars within the species (Salt *et al.*, *Biotechnology*, 13:468-474, 1995). The Office Action also argues that Guerinot *et al.* (*Plant Physiology*, 2001, 125:164-167) suggest that it is unlikely that the regulation of a single gene will be sufficient to convert non-metal accumulators into metal accumulators and that the state of the prior art, as evidenced by Goldsborough (1999, *Phytoremediation in a Contaminated Soil and Water*; CRC Press, Boca; pp. 221-233) teaches transformed *Arabidopsis* plants that did not provide increased heavy metal accumulation as compared to control plants. Therefore, the Office Action argues that, given the lack of guidance in the specification or in the prior art regarding a transformed plant capable of phytoremediation as result of expressing one or more of SEQ ID NO: 1, 3, or 4, the claimed invention is not enabled throughout the broad scope.

Applicants respectfully submit that the references relied upon in establishing the rejection of record are not relevant to the claimed invention and that a *prima facie* case demonstrating that the claimed invention is not enabled has not been established by the Patent Office, particularly as Figures 3 and 4 demonstrate the expression of the claimed polypeptide in onion cells (see, particularly, Example 1, Figure 3(h)(providing evidence of targeting of fusion proteins within the scope of the claims to the cell wall of onion cells) and Figure 4(c)).

Furthermore, Applicants respectfully submit that the transgenic plants expressing a polypeptide comprising SEQ ID NO: 4 or SEQ ID NO: 1 are capable of accumulating heavy metals and that the as-filed specification is enabled for such transgenic plants and methods of using such transgenic plants. As evidence in this regard, Applicants submit the Master's thesis authored by a

graduate student (Brook K. Nelson and submitted in an Information Disclosure Statement with this response) who conducted research in the laboratory of Dr. Mullin under her guidance and direction. Applicants note that Dr. Mullin is one of the inventors listed on this application. As noted in the Abstract of that thesis:

Transient expression of AgNt84-GFP fusion protein in onion epidermal cells confirmed that AgNt84 is targeted to the endoplasmic reticulum. The fusion protein was not found in the cytosol. Expression of AgNt84-GFP with an HDEL retention tag resulted in the accumulation of the fusion protein at what appear to be the plasmodesmata as well as its presence in the endoplasmic reticulum. The presence of AgNt84-GFP in the endoplasmic reticulum is consistent with the pathway that a protein targeted to the plasma membrane would follow. However, the possibility that the protein may be targeted to other organelles or remain in the endoplasmic reticulum remains. To determine the ability of AgNt84 to bind metal *in vivo*, three hydroponically grown tobacco lines transgenic for AgNt84 and wild-type tobacco plants were incubated in 5mM MES buffer containing 200 μ M Cd(NO₃)₂ · 4H₂O. The amount of cadmium in the plants at day 1 and day 3 was analyzed by inductively coupled argon plasma spectrometry (ICP). Two of the transgenic tobacco lines tested had significantly more cadmium in the roots than wild-type tobacco at day 1 and day 3. One of these, T10, was a transgenic line expressing AgNt84, and the expression of AgNt84 in the other tobacco lines remains to be confirmed. Histochemical staining of tobacco tissue using dithizone supported ICP measurements of cadmium content.

Thus, it is clear that: a) transgenic plants expressing a polypeptide comprising SEQ ID NO: 1 or 4 (AgNt84) are capable of accumulating heavy metals, such as cadmium; b) that such transgenic plants are enabled by the as-filed application; and c) methods of bioremediation or phytoremediation of sites contaminated with heavy metals are enabled by the as-filed application. Accordingly, reconsideration and withdrawal of the rejection of record is respectfully requested.

It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position. Applicants expressly reserve the right to pursue the invention(s) disclosed in the subject application, including any subject matter canceled or not pursued during prosecution of the subject application, in a related application.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



Frank C. Eisenschenk, Ph.D.
Patent Attorney
Registration No. 45,332
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

FCE/sl

Attachments: Submission of Sequence Listing and Statement
New pages 1-4 (Sequence Listing)
Supplemental Information Disclosure Statement